

CLAIMS

1. A method for analysing a heterogeneous sample of proteins, peptides or fragments thereof, the method comprising –

5 (a) separating the heterogeneous sample of proteins, peptides or fragments thereof into heterogeneous classes by binding members of each class to a spaced apart defined location on an array, wherein members of each class have a motif common to that class; and

10 (b) characterising the proteins, peptides or fragments thereof in each class.

2. A method according to Claim 1 wherein the heterogeneous sample of proteins or peptides is an extract of the total protein content of a cell or tissue type.

- 15 3. A method according to Claim 1 or 2 wherein, prior to performing step (a) of Claim 1, the heterogeneous sample of fragments is formed by fragmenting a heterogeneous sample of proteins or peptides.

4. A method according to Claim 3 wherein the fragmenting is performed by chemical or enzymatic cleavage.

- 20 5. A method according to Claim 3 or 4 wherein the fragmenting is performed using a sequence-directed cleavage mechanism.

6. A method according to any one of Claim 3 to 5 wherein the fragmenting is performed by digestion of the heterogeneous sample of proteins or peptides with trypsin.

7. A method according to any preceding claim wherein the motif in each protein, peptide or fragment thereof is at the same location in each protein, peptide or fragment thereof, relative to the C-terminus, the N-terminus, or an internal feature.
- 5 8. A method according to any preceding claim wherein the sample is a heterogeneous sample fragments of proteins or peptides and the motif in each fragment is at the same location in each fragment, relative to the site of cleavage.
- 10 9. A method according to any preceding claim wherein the motif in each protein, peptide or fragment thereof is three, four, five, six or more amino acids in length.
- 15 10. A method according to any preceding claim wherein the motif contains three, four or five variable amino acids, the other amino acids in the motif being constant between all proteins, peptides or fragments thereof.
11. A method according to any preceding claim wherein the motif is at the C-terminus.
12. A method according to one of Claims 1 to 10 wherein the motif is at the N-terminus.
- 20 13. A method according to any preceding claim wherein the array comprises a number of different types of binding molecule, each type immobilised at a spaced apart defined location on the array, wherein each type of binding molecule is capable of binding specifically to a defined motif as defined in any preceding claim and wherein different types of binding molecule have different binding specificities.
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14. A method according to Claim 13 wherein the number of different types of binding molecule provided on the array is suitable to capture at least 10% of the proteins or peptides in the unfragmented sample or, where the sample is a heterogeneous sample fragments of proteins or peptides, at least one fragment from at least 10% of the proteins or peptides in the unfragmented sample.
15. A method according to Claim 13 wherein the number of different types of binding molecule provided on the array is suitable to capture at least 50% of the proteins or peptides in the unfragmented sample or, where the sample is a heterogeneous sample fragments of proteins or peptides, at least one fragment from at least 50% of the proteins or peptides in the unfragmented sample.
16. A method according to Claim 13 wherein the number of different types of binding molecule provided on the array is suitable to capture substantially 100% of the proteins or peptides in the unfragmented sample or, where the sample is a heterogeneous sample fragments of proteins or peptides, at least one fragment from substantially 100% of the proteins or peptides in the unfragmented sample.
17. A method according to any one of Claims 13 to 16 wherein the array has at least about 10, 50, 100, 150, 200, 250, 300, or more different types of binding molecules provided thereon.
18. A method according to any one of Claims 13 to 17 wherein at least one type of the binding molecules is an antibody or a fragment or variant thereof, such as Fv, scFv or Fab.
19. A method according to any one of Claims 13 to 18 wherein at least one of the types of the binding molecules is an aptamer.

20. A method according to any one of Claims 13 to 19 wherein at least one of the types of the binding molecules is a polynucleotide.
21. A method according to any one of the previous claims wherein step (b) of Claim 1 comprises characterising bound proteins, peptides or fragments thereof at the defined and discrete locations on the array.
22. A method according to any one of the preceding claims wherein step (b) of Claim 1 comprises determining the mass of proteins, peptides or fragments thereof in the heterogeneous classes.
23. A method according to Claim 19 wherein step (b) of Claim 1 further comprises determining the abundance of proteins, peptides or fragments thereof of different mass in the heterogeneous classes.
24. A method according to any one of the preceding claims wherein step (b) of Claim 1 comprises characterising the proteins, peptides or fragments thereof in the heterogeneous classes by desorption mass spectrometry or collision induced dissociation mass spectrometry.
25. A method according to any preceding claim wherein the information derived from step (b) of Claim 1 is used to determine the identity of the parent protein or peptide in the unfragmented heterogeneous sample from which a detected peptide fragment is derived.
26. A method according to any preceding claim wherein the information derived from step (b) of Claim 1 is used to determine the abundance of a protein or peptide in the heterogeneous sample.
27. A method for identifying differences in composition between two or more heterogeneous fragmented or unfragmented samples of proteins, peptides or fragments thereof comprising analysing each

sample by the method according to any one of the preceding claims and comparing the results, thereby to identify any differences.

28. A method for identifying a disease-related protein or peptide comprising identifying differences between two or more samples by the method of Claim 27, wherein at least one of the samples analysed is derived from an individual with the disease and another one of the samples analysed is derived from a individual without the disease.

29. A method of diagnosing the disease state of an individual comprising analysing an *ex vivo* sample taken from the individual by a method according to anyone of Claims 1 to 26 and determining whether the results correspond with a disease-related protein or peptide identified by the method of Claim 28.

30. An array suitable for use in a method as defined in any one of the preceding claims, comprising a number of different types of binding molecule, each type immobilised at a defined and discrete location on the array, wherein each type of binding molecule is capable of binding specifically to a motif as defined in any one of Claims 7 to 12 and wherein the different types of binding molecule have different binding specificities.

31. An array according to Claim 30 wherein the number of different types of binding molecule provided on the array is such that, when a heterogeneous sample of proteins, or peptides or fragments thereof, is applied to the array, at least 10%, 50% or substantially 100% of the proteins or peptides in the sample or, where the sample is a heterogeneous sample of fragments of proteins or peptides, at least one fragment from at least 10%, 50% or substantially 100% of the

proteins or peptides in the unfragmented sample is captured on the array.

32. An array according to Claim 30 or 31 wherein the number of different types of binding molecule provided on the array is at least about 10, 50, 100, 150, 200, 250, 300, or more.

33. An array according to any one of claims 30 to 32 wherein at least one of the binding molecules is as defined in any one of Claims 18 to 20.

34. A method of producing an array suitable for use in a method according to any one of Claims 1 to 29 comprising –

(a) providing a library of different types of binding molecule, each type being capable of binding specifically to a motif as defined in any one of Claims 7 to 12 and the different types having different binding specificity; and

(b) immobilising the binding molecules on an array such that different types of binding molecule are immobilised at defined and discrete locations.

35. A method according Claim 34 wherein the library of different types of binding molecules comprise at least one type of binding molecule as defined in any one of Claims 18 to 20.

36. An array obtainable by the method of Claim 34 or 35.

37. A system for analysing a heterogeneous sample of proteins or peptides, the system comprising an array according to any one of Claims 30 to 36 and a data carrier comprising information on the

identity and/or binding property and position of each different type of binding molecule on the array.

38. Use of an array or system according to any one of Claims 30 to 37 to analyse one or more heterogeneous samples of proteins, peptides and/or fragments thereof.

39. Use according to Claim 38 to identify a disease-related protein by analysing at least one *ex vivo* sample derived from an individual with the disease and at least one other *ex vivo* sample derived from a individual without the disease.

40. A library of at least about 10, 50, 100, 150, 200, 250, 300, or more different types of binding molecule, each type being capable of binding specifically to a motif as defined in any one of Claims 7 to 12 and the different types having different binding specificities.

41. A library according to Claim 40 comprising at least one type of binding molecule as defined in any one of Claims 18 to 20.

42. A method for making a library of binding molecules comprising –

(a) providing, as a first component, a selector peptide comprising a motif as defined in any one of Claims 7 to 12;

(b) providing, as a second component, a source of candidate binding molecules;

(c) combining the first and second components; and

(d) identifying candidate binding molecules that are capable of specifically binding to the motif of the selector peptide in the first component.

43. A library of at least about 10, 50, 100, 150, 200, 250, 300, or more different types of binding molecules obtainable by the method of Claim 42.
- 5 44. Use of a library of binding molecules as defined by any one of Claims 40, 41 or 43 to produce an array as defined by any one of Claims 30 to 33 or 36.
45. A data carrier comprising information obtainable by a method according to any one of Claims 1 to 29.
- 10 46. An electronic data processing system comprising a data carrier according to Claim 45 and means of comparing information obtainable from the analysis of different samples by a method according to any one of Claims 1 to 29.
- 15 47. Use of a pharmaceutical agent in the manufacture of a medicament for treating an individual identified as being in need thereof by a method according to Claim 29.
48. Method of treating an individual identified as being in need thereof by a method according to Claim 29 comprising administering an effective amount of a pharmaceutical agent appropriate to the disease state of the individual.
- 20 49. A method for making a library of binding molecules comprising –
- (a) providing, as a first component, a selector peptide comprising a motif as defined in any one of Claims 7 to 12;
 - (b) providing, as a second component, a source of candidate binding molecules;
 - 25 (c) combining the first and second components;

(d) identifying candidate binding molecules that are capable of specifically binding to the motif of the selector peptide in the first component;

5 (e) immobilising the binding molecules identified in step (d) on an array such that different types of binding molecule are immobilised at defined and discrete locations

10 (f) providing a heterogeneous sample of proteins, peptides or fragments thereof, which sample comprises proteins, peptides or fragments thereof each having a motif that is bound by a binding molecule immobilised in step (e);

(g) separating the heterogeneous sample of proteins, peptides or fragments thereof into heterogeneous classes by binding members of each class to the binding molecules immobilised in step (e); and

15 (h) characterising the proteins, peptides or fragments thereof in each class.